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due to applying the electric field can be minimized, and a high electric field can be formed, since a strong electric field area is localized at a significantly small region; (3) as the dielectrophoretic force is a force proportional to the electric field gradient, the force is understood as independent on the polarity of the applied voltage, and thus works under an AC electric field in a similar way to a D.C. electric field, and therefore if a high frequency A.C is employed, an electrode reaction (electrolytic reaction) in an aqueous solution can be suppressed, so that the electrodes themselves can be integrated in the channel (sample flow path); (4) improvement in a detection sensitivity can be expected, since there is no restriction to a chamber volume of the detection component as in capillary electrophoresis, and the like.

(Page 5, lines 12-19): However, reports on separation methods with conventional dielectrophoretic forces as described above are limited to separating particles having a low solubility in a solution, relative to DNAs and proteins, such as various cells and latex particles, or otherwise only capturing a single (one kind of) DNA or protein, and any report has not been presented yet on separation of respective molecules from solutions in which are dissolved two or more kinds of biological component molecules, in particular, such as for example DNAs and proteins.

(Page 6, lines 11-14): It is also an object of the present invention to provide a method for separating each other two or more kinds of molecules dissolved in a solution , by using dielectrophoretic forces , such separation having so far been impossible.

(Page 9, line 22 to page 11, line 9): More specifically, the present invention relates to a method for detecting a molecule to be measured in a sample, which comprises reacting a liquid sample, in which a "molecule to be measured" is dissolved, and a solution, in which a "substance specifically binding to the molecule to be measured" is dissolved, to obtain a solution in which a complex substance of the "molecule to be measured" and the "substance specifically binding to the molecule to be measured", and the "substance specifically binding to the molecule to be measured" which is not involved in the reaction are dissolved, placing the solution under a nonuniform electric field having an electric field strength of 500 KV/m or higher, the field being formed by electrodes which have a structure capable of forming a horizontally and vertically ununiform electric field, separating the complex substance from the "substance specifically binding to the molecule to be measured" which is not involved in the reaction, and measuring the "substance specifically binding to the molecule to be measured" in the complex substance, or the the "substance specifically binding to the molecule to be measured" which is not involved in the reaction; and a method for measuring a substance to be measured in a sample comprising, reacting a liquid sample containing a "molecule to be measured", a "molecule to be measured labeled by a labeling substance", and a "substance specifically binding to the molecule to be measured" to obtain a solution containing a complex substance of the "molecule to be measured labeled by a labeling substance" and the "substance specifically binding to the molecule to be measured", a complex substance of the " molecule to be measured" and the "substance specifically binding to the molecule to be measured", and the "molecule to be measured labeled by a labeling substance which is not involved in the reaction,placing

the obtained solution under a nonuniform electric field having an electric field strength of 500 KV/m or higher, the field being formed by electrodes which have a structure capable of forming a horizontally and vertically ununiform electric field, separating the complex substance of the "molecule to be measured labeled by a labeling substance" and the "substance specifically binding to the molecule to be measured" from the "molecule to be measured labeled by a labeling substance" which is not involved in forming the complex, and then measuring the "molecule to be measured labeled by a labeling substance" in the complex substance or the "molecule to be measured labeled by a labeling substance which is not involved in forming the complex substance to determine the amount of the molecule to be measured in the sample based on the results.

(Page 14, line 16 to page 17, line 1): Samples to which the present invention can be applied include samples derived from living body such as body fluids including serum, plasma, cerebrospinal fluid, synovial fluid, lymph, etc., excreta including urine, feces, etc., and treated materials thereof. Treated materials include diluted solutions of these samples derived from a living body in water, buffers, or the like, or those reconstituted by appropriately dissolving or suspending molecules as described below from these body-derived samples in water, buffers, or the like. Samples to which the present invention is applied also include those containing the above described molecules which are chemically synthesized .

(Page 17, line 6 to page 18, line 9): A "substances capable of changing

dielectrophoretic properties" in the present invention (also referred to a separation improving substance) includes a substance which, by binding to a specific molecule (molecule to be measured) to form a complex with the specific molecule, causes differences in behavior to dielectrophoretic operation between the specific molecule and the other co-existing substances (molecules not to be measured, for example, one or more kinds of substances which are not involved in the formation of the complex): for example 1) a substance which can cause a result that any one of both is captured on the dielectrophoresis electrode and the others are not captured, and more specifically, a substance which can provide changes in the movement speed of the specific molecule and the other co-existing substances, for example, in the case of employing a so-called dielectrophoretic chromatography apparatus (Field Flow Fractionation apparatus) in which separation is carried out as described below with the interaction between dielectrophoretic forces caused by the molecules in the electric field and the molecular movement, and more preferably, a substance by which any one of these can be captured on the electrode and the others can be passed through on the dielectrophoresis electrode without being captured on the electrode; or 2) a substance which can cause a result that any one of both receives negative dielectrophoretic forces and the others receive positive dielectrophoretic forces, and more specifically, a substance which, for example, can allow only the specific molecule to gather at a particular position on the dielectrophoretic electrode, and more preferably, a substance which can allow any one of these to gather at a strong electric field strength region on the dielectrophoresis electrode by positive dielectrophoretic forces and the others to gather at a weak electric field strength region on the dielectrophoresis

electrode by negative dielectrophoretic forces; or the like.

(Page 19, lines 1 to 13): A "substance binding to a specific molecule" which can be used in the present invention may not be limited in particular and includes a substance which, from a "specific molecule" in a sample, can form a complex substance of the "specific molecule", a "substance binding to the specific molecule" and a "specific substance capable of changing dielectrophoretic properties", and does not substantially form a complex substance of "molecules other than the specific molecule", the "substance binding to the specific molecule" and the "specific substance capable of changing dielectrophoretic properties". In short, so long as the substance does not form the latter complex substance of the above-mentioned three substances , it can be used for this purpose even if it binds to molecules other than the "specific molecule". Actually, a "substance specifically binding to the specific molecule" is preferably used.

(Page 35, line 6 to page 36, line 8): In the case where the separation is carried out by method (2) of Separation Method-2 described above, the specific molecule or the other molecule can be collected respectively, under conditions where the separation improving substance and the specific molecule bound to the separation improving substance have positive dielectrophoretic forces and the molecules other than the specific molecule have negative dielectrophoretic forces, by collecting at first a mobile phase which contains the molecules other than the specific molecule having negative dielectrophoretic forces and moving without being captured at a particular position on the electrode, and after that,

collecting a washed solution which contains the specific molecule by moving the specific molecule having positive dielectrophoretic forces which is captured at a particular position on the electrode during applying the electric field by ceasing from applying the electric field and washing the electrode with an appropriate buffer usually employed in the art, water, or the like. Alternatively, the specific molecule or the other molecule can be collected respectively , under conditions where the molecules other than the specific molecule have positive dielectrophoretic forces and the separation improving substance and the specific molecule bound to the separation improving substance have negative dielectrophoretic forces, by collecting at first a mobile phase which contains the specific molecule having negative dielectrophoretic forces and moving without being captured at a particular position on the electrode, and after that, collecting a washed solution which contains the molecules other than the specific molecule by moving the molecules having positive dielectrophoretic forces and having been captured at a particular position on the electrode during applying the electric field by ceasing from applying the electric field and washing the electrode with an appropriate buffer usually employed in the art, water, or the like.

(Page 41, lines 9-14): When a solution as described previously has a high conductivity, Joule heat generates by the current flowing in the solution as the voltage is applied, resulting in possibilities of boiling the solution. Therefore, it is preferable that the solutions are used with appropriate adjustment such that the conductivity is usually in the range of not more than 10 mS/cm, preferably not more than 200 μS/cm.

(Page 68, lines 17-21): In the above-mentioned methods, the nucleotide probe and buffers can be selected appropriately according to methods known per se. Method for preparing a nucleotide probe and unknown genes denatured to the single strand, annealing conditions, and the like can be performed according to methods known per se.

(Page 75, line 17 to page 76, line 1): Until now, it is impossible to separate a complex of biotin and a fluorescein-labeled anti-biotin antibody from an unreacted fluorescein-labeled anti-biotin antibody by dielectrophoretic chromatography and the detection of a complex with biotin has not been achieved, because there is no difference in dielectrophoresis separation between the complex and the unreacted antibody to a sufficient extent. The above-mentioned results indicate that applications of a separation improving substance can permit to detect quantitatively by dielectrophoretic chromatography, molecules which have not been detected until now.

(Page 77, lines 14-16): After the antigen-antibody reaction was completed, the reaction solutions were diluted 100 times with distilled water, and the resultants were subject to the dielectrophoretic separation.

(Page 80, lines 4-6): After the antigen-antibody reaction was completed, the reaction solutions were diluted to 100 times with distilled water to and the resultants were subjected to dielectrophoresis.

(Page 80, lines 10-14): The results are shown in Figure 11. It can be found from Figure 11 that a good quantitativeness is obtained within the range of the presence of AFP. From this finding, it is understood that, if serum is used as samples, components in the serum do not affect dielectrophoresis to a great extent, and the detection of a protein to be measured in serum can be achieved.

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(Page 82, lines 5-9): In SSC buffer, 0.05 % (w/v) of the 2kb λDNA probe immobilized latex beads was added to the labeled singled-stranded λDNA and T7 DNA to the final concentration of 20μg/ml, and hybridization was carried out at 68 °C for 18 hours. The sample solution after the hybridization reaction was diluted 100 times with distilled water, and subjected to dielectrophoresis.

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(Page 90, line 17 to page 91, line 11): In this Example, taking account of the event where all the λ DNA are captured and the oligonucleotide is not captured at all, the capture ratio is equal to the percentage of the fluorescence amount derived from the λ DNA occupied in the fluorescence amount of a whole sample. That is, Sample 1 (a sample having a mixing ratio of 0:1 of the labeled oligonucleotide and λ DNA) should give a capture ratio of 100 %, Sample 2 (a sample having a mixing ratio of 1:1 of the labeled oligonucleotide and λ DNA) should give a capture ratio of 1/(1+1) = 50 %, Sample 3 (a sample having a mixing ratio of 5:1 of the labeled oligonucleotide and λ DNA) should give a capture ratio of 1/(1+5) = 16.7 %, and Sample 4 (a sample having a mixing ratio of 1:0 of the labeled oligonucleotide and λ DNA) should give a capture ratio of 0 %.

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